



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/05, 9/00, A61P 27/06		A2	(11) International Publication Number: WO 00/62769 (43) International Publication Date: 26 October 2000 (26.10.00)																		
<p>(21) International Application Number: PCT/US00/09503</p> <p>(22) International Filing Date: 7 April 2000 (07.04.00)</p> <p>(30) Priority Data: 60/130,133 20 April 1999 (20.04.99) US</p> <p>(71) Applicant (for all designated States except US): ALCON LABORATORIES, INC. [US/US]; Mail Code Q-148, 6201 South Freeway, Fort Worth, TX 76134-2099 (US).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): SHADE, Debra, L. [US/US]; Apartment 132, 5021 Timbercreek Drive, Arlington, TX 76017 (US). PANG, Iok-Hou [US/US]; 125 Starbridge Lane, Grand Prairie, TX 75052 (US). CLARK, Abbot, F. [US/US]; 5603 Rachel Court, Arlington, TX 76017 (US).</p> <p>(74) Agents: MAYO, Michael, C. et al.; Alcon Laboratories, Inc., R & d Counsel, Mail Code Q-148, 6201 South Freeway, Fort Worth, TX 76134-2099 (US).</p>		<p>(81) Designated States: AU, BR, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>																			
<p>(54) Title: USE OF AP-1 ACTIVATORS TO TREAT GLAUCOMA AND OCULAR HYPERTENSION</p> <p>(57) Abstract</p> <p>Compositions comprising AP-1 activators and methods of use for treating glaucoma and ocular hypertension are disclosed.</p> <p>Effect of tert-Butylhydroquinone (10 μM) on Flow Rate in Human Ocular Perfusion Organ Culture [Ciliary Body Intact]</p> <table border="1"> <caption>Data from Figure: Effect of tert-Butylhydroquinone (10 μM) on Flow Rate in Human Ocular Perfusion Organ Culture</caption> <thead> <tr> <th>Time After Treatment (h)</th> <th>Vehicle (%)</th> <th>tBHQ (%)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>100</td> <td>100</td> </tr> <tr> <td>24</td> <td>85*</td> <td>100</td> </tr> <tr> <td>48</td> <td>75</td> <td>100</td> </tr> <tr> <td>72</td> <td>65*</td> <td>110</td> </tr> <tr> <td>96</td> <td>55*</td> <td>120*</td> </tr> </tbody> </table>				Time After Treatment (h)	Vehicle (%)	tBHQ (%)	0	100	100	24	85*	100	48	75	100	72	65*	110	96	55*	120*
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Use of AP-1 Activators to Treat Glaucoma and Ocular Hypertension

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The present invention relates to the treatment of glaucoma and ocular hypertension. In particular, the present invention relates to the use of compounds that increase the activity of Activator Protein-1 (AP-1) to treat glaucoma and ocular hypertension.

10 Background of the Invention

Glaucoma is a progressive disease which leads to optic nerve damage and, ultimately, total loss of vision. The causes of this disease have been the subject of extensive studies for many years, but are still not fully understood. The principal symptom of, and/or risk factor for, 15 the disease is elevated intraocular pressure (IOP) or ocular hypertension.

The reasons why IOP is increased in glaucoma patients are not fully understood. It is known that elevated IOP can be at least partially controlled by administering drugs which either reduce the production of aqueous humor within the eye, such as beta-blockers and carbonic anhydrase inhibitors, or increase the flow of aqueous humor out of the eye, such as cholinergic 20 agonists and sympathomimetics.

All types of drugs currently being used to treat glaucoma have potentially serious side effects. Cholinergic agonists such as pilocarpine can cause blurring of vision and other ocular side effects, which may lead either to decreased patient compliance or to termination 25 of therapy. Systemically administered carbonic anhydrase inhibitors can also cause serious side effects, such as nausea, dyspepsia, fatigue, and metabolic acidosis which can affect patient compliance and/or necessitate the withdrawal of treatment. Moreover, some beta-blockers have been known to be associated with pulmonary side effects attributable to their effects on beta-2 receptors in the pulmonary tissue. Sympathomimetics cause tachycardia, arrhythmia and hypertension. There is therefore a continuing need for new therapies which 30 control elevated IOP associated with glaucoma.

Activator protein-1 (AP-1) is a dimeric gene transcription promoter comprised of subunit proteins which are the products of at least three different proto-oncogene families: the Jun (c-Jun, v-Jun, JunB, JunD), Fos (c-Fos, v-Fos, FosB, FosB2, Fra-1, Fra-2) or activating transcription factor (B-ATF, ATF2, ATF3/LRF1) families. Uncomplexed monomers and

homo- and hetero-dimers of these protein subunits have been observed in a variety of mammalian and non-mammalian tissues (Foletta et al., *Transcriptional regulation in the immune systems: all roads lead to AP-1*, J. Leukoc Biol., volume 63, pages 139-152 (1998); and Karin et al., *AP-1 function and regulation*, Curr. Opin. Cell Biol., volume 9, pages 240-246 (1997)).

5 AP-1 binds to specific DNA sequences within enhancer regions of many genes (e.g., TPA (12-O-tetradecanoylphorbol-13-acetate) response elements (TREs) or cyclic AMP response elements (CREs)) and promotes the activation of the particular gene. Examples of genes which contain AP-1 consensus sequences in their enhancer regions include the genes for
10 SV40 and human metallothionein IIA (Lee et al., *Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40*, Nature, volume 325, pages 368-372). The binding affinity of AP-1 for the various response elements appears to depend on the specific protein subunit dimer complex. Dimers composed of Jun/Jun or Jun/Fos generally bind TREs, whereas ATF/ATF or Jun/ATF dimers
15 preferentially bind CREs (Whitmarsh et al., *Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways*, J. Mol. Med., volume 74, pages 589-607 (1996); and Karin et al., *Current Opin. Cell Biol.*, volume 9, pages 240-246 (1997)). The biological consequences of AP-1 mediated gene transcription may also vary, depending upon the dimer composition. For example, induction of murine glutathione-S-transferase genes is
20 apparently mediated by a Fos/Jun heterodimer which binds at least one TRE sequence within the gene's antioxidant response element (Ainbinder et al., *Regulatory mechanisms involved in activator-protein-1 (AP-1)-mediated activation of glutathione-S-transferase gene expression by chemical agents*, Eur. J. Biochem., volume 243, pages 49-57 (1997); Xie et al., *ARE- and TRE-mediated regulation of gene expression*, J. Biol. Chem., volume 270, pages 6894-6900
25 (1995)), and a c-Jun/ATF-2 complex has been shown to bind to a CRE involved in activation of the T-cell gene for tumor necrosis factor-alpha (Foletta et al., J. Leukoc. Biol., volume 63, pages 139-152 (1998)).

Several AP-1 Activators have been reported previously, including β -naphthoflavone and *tert*-butylhydroquinone (tBHQ) (Ainbinder et al., Eur. J. Biochem., volume 243, pages 49-57 (1997); Ainbinder et al., *Signaling pathways in the induction of c-fos and c-jun proto-oncogenes by 3-methylcholanthrene*, Receptors and Signal Transduction, volume 7, pages 279-289 (1998); and Oazki et al., *The comparative effects of haloperidol, (-)-sulpiride, and*

SCH23390 on c-fos and c-jun mRNA expressions, and AP-1 DNA binding activity, Eur. Neuropsychopharmacol., volume 7, pages 181-187 (1997)). Nowhere in the art, however, has it been taught or suggested that AP-1 activators may be useful in treating glaucoma or ocular hypertension.

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Summary of the Invention

The present invention is directed to compositions and methods of use for the treatment of glaucoma and ocular hypertension. More specifically, the invention is directed to compositions comprising AP-1 activators and methods of use.

Preferred methods involve the topical administration of compositions comprising tBHQ.

Description of the Drawing

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Figure 1 is a graph showing the effects of tBHQ on IOP in a human ocular perfusion organ culture model.

Detailed Description of the Invention

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The present invention is directed to compositions and methods of use for the treatment of glaucoma and ocular hypertension.

The compounds useful in the present invention compositions and methods are AP-1 Activators. While not intending to be bound by any theory, it is believed that the stimulation of AP-1 activity leads to the transcription of mRNAs and corresponding translation of proteins useful in effecting the maintenance or lowering of IOP. As used herein, "AP-1 Activator" refers to those molecules which lower IOP via AP-1 mediated transcription. Such activators may include agents which (1) increase the formation and expression of proteins comprising AP-1 complex(es), (2) enhance the formation of such complexes, or (3) promote binding of an AP-1 complex to regulatory/promoter sites on cellular genes. Examples of AP-1 Activators include β -naphthoflavone, *tert*-butylhydroquinone (tBHQ), sulpiride, haloperidol, and 3-methylcholanthrene. The most preferred AP-1 Activator is tBHQ.

Other AP-1 Activators may be elucidated by methods known in the art. For example, an increase in the level of DNA-bound AP-1 complex, as assessed by electrophoretic mobility shift assay, is an accepted means for detection of activated AP-1 activity. Such DNA-binding can be determined by the following protocol:

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DNA-Binding Assay:

Target cellular tissue (e.g., human trabecular meshwork tissue and/or cultured cells) is treated with or without an AP-1 Activator candidate(s). Nuclear extracts of the cells are then 10 prepared according to the method of Schreiber et al., *Rapid detection of octamer binding proteins with 'mini-extracts' prepared from a small number of cells*, Nucleic Acids Res., volume 17, page 6419 (1989). Electrophoretic mobility shift assays are then carried out with the nuclear extracts as described by Ainbinder et al., *Regulatory mechanisms involved in activator-protein-1 (AP-1)-mediated activation of glutathione-S-transferase gene expression by chemical agents*, Eur. J. Biochem., volume 243, pages 49-57 (1997). Briefly, extracts are 15 incubated with a ³²P-labelled AP-1 oligonucleotide probe and the resulting complexes are then separated by non-denaturing acrylamide gel electrophoresis. Levels of DNA binding activity can then be determined from analysis of autoradiograms of the dried gels. By using this method, compounds such as tBHQ have been demonstrated to be AP-1 Activators.

20 Other AP-1 Activators may be identified by routine methods known in the art. For example, methods disclosed in the following publications may be useful in elucidating other AP-1 Activators of the present invention:

- (1) Ainbinder et al., *Regulatory mechanisms involved in activator-protein-1 (AP-1)-mediated activation of glutathione-S-transferase gene expression by chemical agents*, Eur. J. Biochem., volume 243, pages 49-57 (1997);
- (2) Ainbinder et al., *Signaling pathways in the induction of c-fos and c-jun proto-oncogenes by 3-methylcholanthrene*, Receptors and Signal Transduction, volume 7, pages 279-289 (1998);
- (3) Ozaki et al., *The comparative effects of haloperidol, (-)-sulpiride, and SCH23390 on c-fos and c-jun mRNA expressions, and AP-1 DNA binding activity*, Eur. Neuropsychopharmacol., volume 7, pages 181-187 (1997); and

(4) Xie et al., *ARE- and TRE-mediated regulation of gene expression*, J. Biol. Chem., volume 270, pages 6894-6900 (1995); the foregoing publications are incorporated herein by reference.

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Example 1

The following example illustrates the effect of an AP-1 Activator, tBHQ, on aqueous outflow facility as demonstrated using a human ocular perfusion organ culture model. Pharmaceutical candidates which maintain or increase outflow facility are believed to be 10 useful in treating or controlling IOP and, hence, glaucoma. The study was performed as follows:

Human donor eyes less than 24 hours *post mortem* were used. The globe was sectioned equatorially followed by the removal of vitreous, lens, zonules, iris, and most of the ciliary body. The anterior segment was then mounted on a perfusion dish, perfused with cell 15 culture medium at a constant hydrostatic pressure of 10 mm Hg (Johnson et al., *Human trabecular meshwork organ culture: A new method*, Invest Ophthalmol Vis Sci., volume 28, pages 945-953 (1987); Erickson-Lamy et al., *Outflow facility studies in the perfused human ocular anterior segment*, Exp. Eye Res., volume 52, pages 723-731 (1991); and Clark et al., *Dexamethasone-induced ocular hypertension in perfusion cultured human eyes*, Invest. 20 Ophthalmol. Vis. Sci., volume 36, pages 478-489 (1995)). The flow rate of the perfusate was measured by weighing the reservoir at predetermined periods. After 2 to 4 days of equilibrium period, one eye of each of the donor pair was perfused with the test compound, t-BHQ (10 μ M), while the other was perfused with vehicle (control). The results are shown in Figure 1 and Table 1, below:

Table 1: Effect of t-BHQ (10 μ M) on Aqueous Outflow Rate in Human Ocular Perfusion Organ Culture

Time after Treatment (Hours)	Flow Rate (% of Time 0) (Mean \pm SEM, n = 6)	
	Vehicle	t-BHQ
-4	100.0 \pm 0.8	97.0 \pm 4.0
0	100.0	100.0
4	92.0 \pm 3.6	105.8 \pm 5.4*
24	92.0 \pm 4.8	102.9 \pm 3.9*
48	86.6 \pm 9.2	105.2 \pm 10.1
72	76.6 \pm 12.5	111.8 \pm 11.8*
96	61.8 \pm 12.1	120.9 \pm 16.4*

5 Note: (1) The basal flow rate at time 0 for vehicle-treated eyes was $3.55 \pm 1.00 \mu\text{L}/\text{min}$, and that for drug-treated eyes was $4.09 \pm 0.63 \mu\text{L}/\text{min}$.
 (2) * represents $p < 0.05$, paired t-test compared to the vehicle-treated eye of the same time point.

10 While not intending to be bound by any theory, it is believed that the AP-1 Activators of the present invention are useful in increasing the outflow facility of a mammal's eye, which leads to maintained or lowered IOP, and thus useful in the treatment of glaucoma or ocular hypertension.

15 The preferred route of administration for the methods of the present invention is topical. The preparation of topical ophthalmic compositions is well known in the art. Generally, topical ophthalmic compositions useful in the present invention will be in the form of a solution, suspension, gel, or formulated as part of a device, such as a collagen shield or other bioerodible or non-bioerodible device. Various excipients may be contained in the topical ophthalmic solutions, suspensions or gels of the present invention. For example, 20 buffers (e.g., borate, carbonate, phosphate), tonicity agents (e.g., sodium chloride, potassium chloride, polyols), preservatives (e.g., polyquaterniums, polybiguanides, benzalkonium chloride), chelating agents (e.g., EDTA), viscosity enhancing agents (e.g., polyethoxylated glycols) and solubilizing agents (e.g., polyethoxylated castor oils, including polyoxyl-35 castor oil (Cremophor EL[®], BASF Corp., Parsippany, NJ); Polysorbate 20, 60 and 80; Pluronic[®] F-

68, F-84 and P-103 (BASF Corp.); or cyclodextrin) may be included in the topical ophthalmic compositions. However, preferable compositions of the present invention will not include preservatives or tonicity agents which are known to adversely affect or irritate the eye.

5 A variety of gels may be useful in topical ophthalmic gel compositions of the present invention, including, but not limited to, carbomers, polyvinyl alcohol-borates complexes, or xanthan, gellan, or guar gums. Topical ophthalmic bioerodible and non-bioerodible devices are known in the art and may be useful in the topical administration of the AP-1 Activators. *See, for example, Weiner, A.L., *Polymeric Drug Delivery Systems For the Eye*, in Polymeric Site-specific Pharmacotherapy, Ed., A.J. Domb, John Wiley & Sons, pages 316-327 (1994).*

10 While the particular ingredients and amounts to be contained in topical ophthalmic compositions useful in the methods of the present invention will vary, particular topical ophthalmic compositions will be formulated to effect the administration of one or more AP-1 Activators topically to the eye.

15 In general, the doses of AP-1 Activators used for the above described purposes will vary, but will be in an amount effective to maintain or lower IOP, or otherwise treat glaucoma or ocular hypertension. As used herein, the term "pharmaceutically effective amount" refers to that amount of an AP-1 Activator administered to a mammal which maintains or lowers IOP, or otherwise ameliorates the glaucomatous condition of the patient. The AP-1 Activators will normally be contained in the compositions described herein in an amount from about 0.00001 to about 2.0 percent weight/volume ("% w/v"), preferably 0.01 to 2% w/v. The compositions of the present invention may be delivered topically to the eye, about one to six times a day.

20 As used herein, the term "pharmaceutically acceptable vehicle" refers to any formulation which is safe, and provides the appropriate delivery for the desired route of administration of an effective amount of at least one AP-1 Activator of the present invention.

25 Preferred formulations of AP-1 Activators combinations of the present invention include the following Examples 2-3:

Example 2

Topical ophthalmic formulation:

Ingredient	Amount (%wt)
AP-1 Activator	0.01 to 2
Phosphate Buffered Saline	1.0
Benzalkonium Chloride	0.01
Polysorbate 80	0.5
Purified water	q.s. to 100%

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Example 3

Topical ophthalmic formulation:

Ingredient	Amount (%wt)
t-HBQ	0.01 to 2
Phosphate Buffered Saline	1.0
Hydroxypropyl- β -cyclodextrin	4.0
Purified water	q.s. to 100%

Example 4

10 Preferred formulation for a topical ophthalmic solution:

Component	Amount (%wt)
t-HBQ	0.01 to 2
Benzalkonium chloride	0.01
HPMC	0.5
Sodium chloride	0.8
Sodium phosphate	0.28
Edetate disodium	0.01
NaOH/HCl	q.s. to pH 7.2
Purified Water	q.s. to 100%

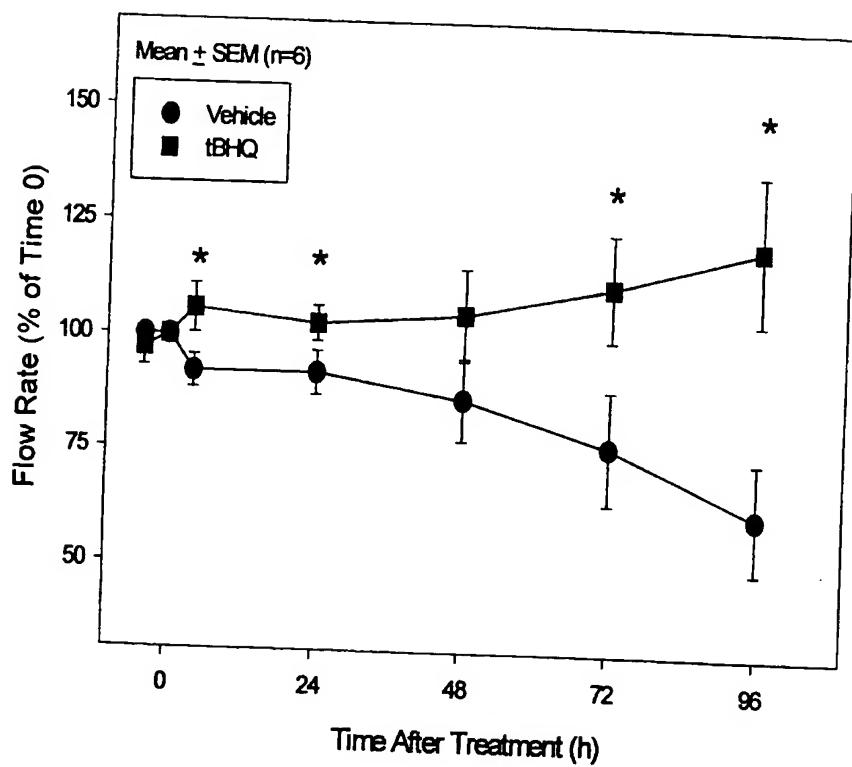
We Claim:

1. A topical composition for treating glaucoma or ocular hypertension comprising an AP-1 Activator and a pharmaceutically acceptable vehicle.
- 5 2. A composition according to Claim 1, wherein the AP-1 Activator is selected from the group consisting of: β -naphthoflavone, *tert*-butylhydroquinone, and sulpiride.
- 10 3. A composition according to Claim 2, wherein the AP-1 Activator is *tert*-butylhydroquinone.
4. A composition according to Claim 1, wherein the composition comprises:
 - 15 about 0.01 to 2.0 % w/v t-HBQ;
 - about 0.28 % w/v sodium phosphate;
 - about 0.8% w/v sodium chloride;
 - about 0.01 % w/v benzalkonium chloride
 - about 0.5 % w/v HPMC;
 - 20 about 0.1 % w/v edetate disodium; and water
5. A method for treating glaucoma or ocular hypertension which comprises administering to a mammal a composition comprising an AP-1 Activator and a pharmaceutically acceptable vehicle.
- 25 6. A method according to Claim 5, wherein the AP-1 Activator is selected from the group consisting of: β -naphthoflavone, *tert*-butylhydroquinone, and sulpiride.
7. A method according to Claim 6, wherein the AP-1 Activator is *tert*-butylhydroquinone.
- 30 8. A method according to Claim 5, wherein the composition comprises:
 - about 0.01 to 2.0 % w/v t-HBQ;

about 0.28 % w/v sodium phosphate;
about 0.8% w/v sodium chloride;
about 0.01 % w/v benzalkonium chloride
about 0.5 % w/v HPMC;
5 about 0.1 % w/v edetate disodium; and water.

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Effect of tert-Butylhydroquinone (10 μ M) on Flow Rate in
Human Ocular Perfusion Organ Culture
[Ciliary Body Intact]



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